ART 34 AMDT

WHAT IS CLAIMED IS:

1. A method of determining an individual's predisposition to breast cancer, development of breast cancer and/or responsiveness to therapy for breast cancer, said method comprising the step of determining a combination of a *bsmI* polymorphism located in the last intron of the vitamin D receptor (VDR) gene or a DNA variant equivalent, or mutation which shows a linkage disequilibrium therewith, and a *FokI* polymorphism in exon I of VDR, or a DNA variant equivalent, or mutation which shows a linkage disequilibrium therewith, whereby said combination of polymorphisms at the VDR gene, or markers in linkage disequilibrium therewith enables a prediction of an individual's predisposition to breast cancer, development of breast cancer and/or responsiveness to therapy for breast cancer.

2. The method of claim 1, wherein the step of determining the vitamin D receptor genotype comprises restriction endonuclease digestion.

3. The method of claim 1, wherein the step of determining the vitamin D receptor genotype comprises hybridizing with allele specific oligonucleotides.

4. The method of claim 2, which further comprises a step, prior to determining the vitamin D receptor genotype, of amplifying a segment of the vitamin D receptor gene using polymerase chain reaction.

5. The method of claim 3, which further comprises a step, prior to determining the vitamin D receptor genotype, of amplifying a segment of the vitamin D receptor gene using polymerase chain reaction.

6. The method of claim 2, wherein at least one endonuclease selected from the group consisting of *BsmI*, *ApaI*, *TaqI*, and isoschizomers thereof is used for determining the vitamin D receptor genotype.

CA 2289769 2000-11-16

ART 34 AMDT

7. The method of claim 2, wherein FokI and isoschizomers thereof are used for analyzing the vitamin D receptor genotype.

8. The method of claim 4, wherein a pair of primers derived from a nucleic acid sequence of the vitamin D receptor gene or flanking said gene is used in the polymerase chain reaction.

9. The method of claim 5, wherein a pair of primers derived from a nucleic acid sequence of the vitamin D receptor gene or flanking said gene is used in the polymerase chain reaction.

10. The method of claim 8, wherein the segment of the vitamin D receptor gene is amplified using a pair of primers as defined as 5'-CAACCAAGAC TACAAGTACC GCGTCAGTGA-3' (SEQ ID NO:1) and 5'-TATCGTGAGT AAGGCAGGAG AGGGAGACC-3' (SEQ ID NO.2).

11. The method of claim 9, wherein the segment of the vitamin D receptor gene is amplified using a pair of primers as defined as 5'-CAACCAAGAC TACAAGTACC GCGTCAGTGA-3' (SEQ ID NO:1) and 5'-TATCGTGAGT AAGGCAGGAG AGGGAGACC-3' (SEQ ID NO:2).

12. The method of claim 8, wherein the segment of the vitamin D receptor gene is amplified using a pair of primers as defined as 5'-AGCTGGCCCT GGCACTGACT CTGCTCT-3' (SEQ ID NO:3) and 5'-ATGGAAACAC CTTGCTTCTT CTCCCTC-3' (SEQ ID NO:4).

13. The method of claim 9, wherein the segment of the vitamin D receptor gene is amplified using a pair of primers as defined as 5'-AGCTGGCCCT GGCACTGACT CTGCTCT-3' (SEQ ID NO:3) and 5'-ATGGAAACAC CTTGCTTCTT CTCCCTC-3' (SEQ ID NO:4).

14. The method of claim 2, wherein the vitamin D receptor

ART 34 AMDT

genotype is determined using a polymorphic variant site in linkage disequilibrium with at least one allelic variant as detected with BsmI, ApaI, and TaqI in the restriction endonuclease digestion.

15. The method of claim 2, wherein the vitamin D receptor genotype is determined using a polymorphic variant site in linkage disequilibrium with at least one allelic variant as detected with FokI in the restriction endonuclease digestion.

16. An assay for screening and selecting an agent which modulates breast cancer predisposition comprising:

a) a recombinant vitamin D receptor gene or functional fragment thereof, which comprises at least one of a *BsmI* and *FokI* polymorphism, or a marker in linkage disequilibrium with said *BsmI* and *FokI* polymorphism; and

b) assaying a function of said vitamin D receptor in the presence of an agent;

wherein an allele or combination of alleles which modulates said function of said vitamin D receptor can be selected, and wherein a modulation of said function of said vitamin receptor is associated with a modulation of said breast cancer predisposition, thereby leading to breast cancer protection or breast cancer predisposition.

17. An assay for screening and selecting an agent which modulates breast cancer predisposition comprising:

a) an expression vector comprising a promoter operably linked to a reporter gene, said promoter comprising a vitamin D response element, said response element affecting the activity of said promoter upon binding thereto of vitamin D, or analog thereof;

b) a cell expressing a chosen allele or combination of alleles of a vitamin D receptor and harboring said vector of a);

ART 34 AMDT

c) submitting said cell to at least one agent; and

d) assaying a level of said reporter gene;

whereby an agent which modulates breast cancer predisposition can be selected when the level of said reporter gene is significantly modulated by the presence of said agent, through its action through the vitamin D receptor.

18. A method for screening and selecting an agent which can modulate breast cancer predisposition comprising

a) selecting a specific allele of the vitamin D receptor (VDR) gene, variant, equivalent, or mutation thereof which shows linkage disequilibrium therewith;

b) assaying a function of said VDR allele of a); and

c) selecting an agent which can modulate breast cancer predisposition,

wherein an agent which modulates VDR function is selected as an agent capable of modulating breast cancer predisposition when said function is significantly different in the presence of said agent, as compared to in the absence thereof.

Sub A' 19. The method of one of claims 1 to 15 or 18, wherein the genotype VDR-BBff is associated with a significant predisposition to breast cancer.

20. The method of claim 18, wherein said assay is a *cis-trans* assay.

Add A2